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Title: Rapid detection of Mycobacterium tuberculosis from clinical specimens using a new target

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Body: Background: Different DNA targets have been introduced for detection of M. tuberculosis. Of these targets, IS6110 has been used frequently. However, certain strains of M. tuberculosis, particularly those identified in Southeast Asia, lack this insertion sequence. Aims and objectives: Cytochrome P450 CYP141 has not been used as target for detection of M. tuberculosis. The aim of this study was to develop a new sensitive and specific PCR method for detection of M. tuberculosis directly from respiratory specimens. Method: The identification of M. tuberculosis isolates was confirmed using standard biochemical tests. Primer 3 plus software was used to design primers from CYP141. The expected size of the amplicon was 173 bp. We collected sputa from 247 suspected patients of different cities of Iran. Results: With the exception of M. tuberculosis complex, no amplification was obtained with DNA from other mycobacteria, potentially pathogenic bacteria in the respiratory tract and human cells. These results give evidence that CYP141 can be used as a target for direct detection of M. tuberculosis from respiratory specimens. The sensitivity of this target in smear positive- culture positive and smear negative-culture positive samples was 92% and 62.5%, respectively, and the specificity of the PCR was 97.8%. Discussion We obtained positive results with a trace amount of template DNA, as little as 1pg. Rv3121 or CYP141 exists in all M. tuberculosis isolates used in this study. The high overall specificity (97.8%) and sensitivity (85.7%) of this target in gene amplification illustrates that CYP141 can be a good target for detection of M. tuberculosis complex from sputum and possibly other clinical specimens.